The 97 kDa Linear IgA Bullous Dermatosis Antigen is not Expressed in a Patient with Generalized Atrophic Benign Epidermolysis Bullosa with a Novel Homozygous G258X Mutation in COL17A1

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The nature and expression pattern of the 97 kDa linear IgA bullous dermatosis antigen (LAD-1) and its role in epidermolysis bullosa have not been fully elucidated. In this study, we examined the expression of LAD-1 in the skin specimens of 70 patients with the various subtypes of epidermolysis bullosa, including simplex (n = 23), junctional (n = 15), and dystrophic variants (n = 32). For immunolabeling, we used two recently developed monoclonal antibodies to LAD-1 whose epitopes were ultrastructurally localized in the lamina lucida between NC16A and carboxyterminal domains of BPAG2, as well as autoantibodies against LAD-1 from the sera of two patients with linear IgA dermatosis. Among the 70 patients, only one patient with generalized atrophic benign epidermolysis bullosa failed to demonstrate LAD-1 expression. Although other major basement membrane components, including laminin 5, BPAG1, plectin, α6 and β4 integrins, as well as type IV and type VII collagens were normally expressed, BPAG2/type XVII collagen was absent from the skin of this patient. Mutation analysis on COL17A1 using polymerase chain reaction amplification, heteroduplex scanning, and direct nucleotide sequencing revealed that this patient was homozygous for a novel nonsense mutation G258X in exon 11, and her parents were heterozygous carriers for this mutation. This is the first mutation located in the intracellular domain of BPAG2, and resides 817 bp upstream from the N-terminal amino acid sequence of LAD-1. These findings indicate that the absent expression of LAD-1 is observed in a BPAG2-deficient generalized atrophic benign epidermolysis bullosa patient with mutations in both alleles of COL17A1, and not in other epidermolysis bullosa subtypes. These findings also support the notion that LAD-1 is a degradation product of BPAG2. ***Key words: basement membrane/bullous pemphigoid/hemidesmosome. J Invest Dermatol 111:887–892, 1998***

Epidermolysis bullosa (EB) is a group of inherited skin diseases that consists of more than 20 clinical variants. The characteristic feature common to each subtype is marked fragility of the skin, with the formation of blisters in response to seemingly minor or insignificant trauma (Fine *et al*, 1991). Conventional classification of EB into three major types, simplex, junctional, and dystrophic, is based on the level of blister formation within the dermal–epidermal basement membrane zone (BMZ) upon ultrastructural examination.

Recent studies have revealed the existence of distinct BMZ proteins, recognized by the circulating autoantibodies in patients with certain autoimmune bullous diseases. Also, mutations in the corresponding genes underlie heritable forms of EB in patients in clinically defined variants. For example, mutations in the gene encoding type VII collagen, a target antigen of autoimmune disease epidermolysis bullosa acquisita (Shimizu *et al*, 1997a), are responsible for dystrophic EB (Uitto and Christiano, 1994); laminin 5, a target molecule for autoantibodies in patients with cicatricial pemphigoid (Domloge-Hultsch *et al*, 1992; Shimizu *et al*, 1995), is the gene/protein system altered in some forms of inherited junctional EB (Uitto *et al*, 1994); and mutations in BPAG2, also known as type XVII collagen, recognized by autoantibodies in patients with bullous pemphigoid (Diaz *et al*, 1990), are responsible for EB in a subgroup of patients known as generalized atrophic benign epidermolysis bullosa (GABEB) (McGrath *et al*, 1995a; Jonkman *et al*, 1996; Gatalica *et al*, 1997; Scheffer *et al*, 1997).

Linear IgA dermatosis is an autoimmune blistering disease that is characterized by subepidermal blister formation with marked neutrophilic inflammation. In this disease, the 97 kDa linear IgA dermatosis antigen (LAD-1), which has been suggested to be synthesized as a 120 kDa precursor protein by keratinocytes (Marinkovich *et al*, 1996), is the major target molecule for IgA class autoantibodies (Zone *et al*, 1990). The absence of LAD-1 and BPAG2 in GABEB patients was first reported in three patients by Pas *et al* (1997), and this was later

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Abbreviations: EB, epidermolysis bullosa; GABEB, generalized atrophic benign epidermolysis bullosa; LAD-1, 97 kDa linear IgA bullous dermatosis antigen.

confirmed in 13 patients by Marinkovich *et al* (1997). Pas *et al* (1997) demonstrated that LAD-1 and BPAG2 are both absent in cultured keratinocytes as well as in the skin of BPAG2-deficient GABEB patients, but their expression returns to normal after repair of the COL17A1 mutation by mitotic gene conversion. Subsequent immunoscreening of 44 patients with junctional forms of EB also revealed the absence of LAD-1 in some patients with the GABEB phenotype (Marinkovich *et al*, 1997). Pas *et al* (1997) also showed that a subset of bullous pemphigoid sera and liner IgA dermatosis sera reacts with LAD-1, but not with BPAG2, thus suggesting that there are unique epitopes on LAD-1.

We recently developed monoclonal antibodies against LAD-1 (Zone *et al*, 1998), and demonstrated by immunoelectron microscopy that the epitopes of both antibodies localized in the lamina lucida corresponding to a region between the NC16A domain and the carboxy-terminal segments of BPAG2 (Ishiko *et al*, in press). In this study, we immunoscreened 70 patients with different subtypes of EB for the expression of LAD-1 using these novel monoclonal antibodies, and elucidated the molecular basis of EB in a patient with absent expression of this molecule.

MATERIALS AND METHODS

Patients and skin samples Fresh skin samples were obtained from a cohort of 70 Japanese patients with various subtypes of EB, who were referred to the Special Clinic for Genetic Counseling on Inherited Skin Diseases of the Keio University Hospital, Tokyo, Japan. Diagnoses included EB simplex in 23 patients, consisting of four Dowling Meara (Nomura *et al*, 1996), two Koebner, 13 Weber-Cockayne, and four EB simplex associated with muscular dystrophy (Pulkkinen *et al*, 1996); junctional EB in 15 patients, including seven Herlitz (Shimizu *et al*, 1997b), seven non-lethal among which three were with typical GABEB phenotype, and one associated with pyloric atresia (Shimizu *et al*, 1996a; Takizawa *et al*, 1997); dystrophic EB in 32 patients, including 20 with recessive and 12 with dominant inheritance (Shimizu *et al*, 1996b). Diagnosis of the EB subtype was based on the clinical, electron microscopic, and immunohistochemical features.

Case report: patient with GABEB associated with absence of LAD-1 and COL17A1/BPAG2 expression One of the 70 patients with EB examined in this study is a 63 y old female with the junctional form of EB presenting with GABEB phenotype. She showed a complete absence of LAD-1 expression in her skin. She had a history of generalized trauma-induced blistering since birth. Examination of her skin revealed blisters, erosions, and areas of thin, cigarette paper-like atrophic depigmented skin at sites of recurrent blistering, but no milia. Her growth had been normal. There was moderate improvement in the severity of blistering with advanced age. She exhibited dystrophic nails and hypoplastic teeth, mild involvement of the mucous membranes, and nonscarring alopecia (**Fig 1*a–f***). Her eyelashes and eyebrows were scanty, and pubic and axillary hair were absent. There was no family history of EB, and her parents were not known to be related.

Antibodies The following antibodies directed against a range of epidermal BMZ-associated antigens were used in immunofluorescence studies of the skin. For LAD-1, we used two newly produced monoclonal antibodies, MoAb 97–1 and MoAb 97–2, that react with LAD-1 but not with BPAG2 by immunoblot. Details of production and characterization of these monoclonal antibodies have been reported elsewhere (Zone *et al*, 1998). Using immunoelectron microscopy, we recently confirmed that the epitopes for these two monoclonal antibodies localized to the lamina lucida and recognized a region between the NC16A domain and carboxy terminal segment of BPAG2 (Ishiko *et al*, 1998). Two sera from patients with linear IgA dermatosis that were previously shown by immunoblot analysis to react with LAD-1 and to bind to the lamina lucida by immunoelectron microscopy (Ishiko *et al*, 1996), were also used for detection of LAD-1. The following monoclonal antibodies were used: for the 180 kDa bullous pemphigoid antigen (BPAG2), monoclonal antibodies D20, 233, and 1D1 (Nishizawa *et al*, 1993); for α6 integrin, monoclonal antibody GoH3 (Sonnenberg *et al*, 1987); for β4 integrin, monoclonal antibody 439–9B (Kennel *et al*, 1989, 1990); for HD1/plectin, monoclonal antibody 121 (Hieda *et al*, 1992); for the 230 kDa bullous pemphigoid antigen (BPAG1), polyclonal antibody S1193 (Tanaka *et al*, 1994); for laminin 5, monoclonal antibody GB3 (Verrando *et al*, 1991) (Sera Laboratory, Cambridge, U.K.); for uncein, monoclonal antibody 19-DEJ-1 (Fine *et al*, 1989, 1990); for type VII collagen, monoclonal antibody LH7.2 (Leigh *et al*, 1987) (Chemicon); for type IV collagen, a commercially available monoclonal antibody (Dakopatts, Denmark).

Immunofluorescence Immunofluorescence was performed as previously described (Shimizu *et al*, 1996a). In brief, 6 μm cryostat sections of skin specimens obtained from the area on and around an existing blister or from a blister that was induced by applying friction, were embedded in OCT and stored frozen at –80°C. They were subsequently incubated at 37°C for 60 min with the primary antibody. After washing with phosphate-buffered saline, the sections were incubated with fluorescein isothiocyanate-conjugated secondary antibodies (Dako, Denmark). After a further washing, the sections were mounted and viewed under a standard Olympus microscope equipped for epifluorescence. Normal mouse, rat, rabbit, or human serum was used as negative controls.

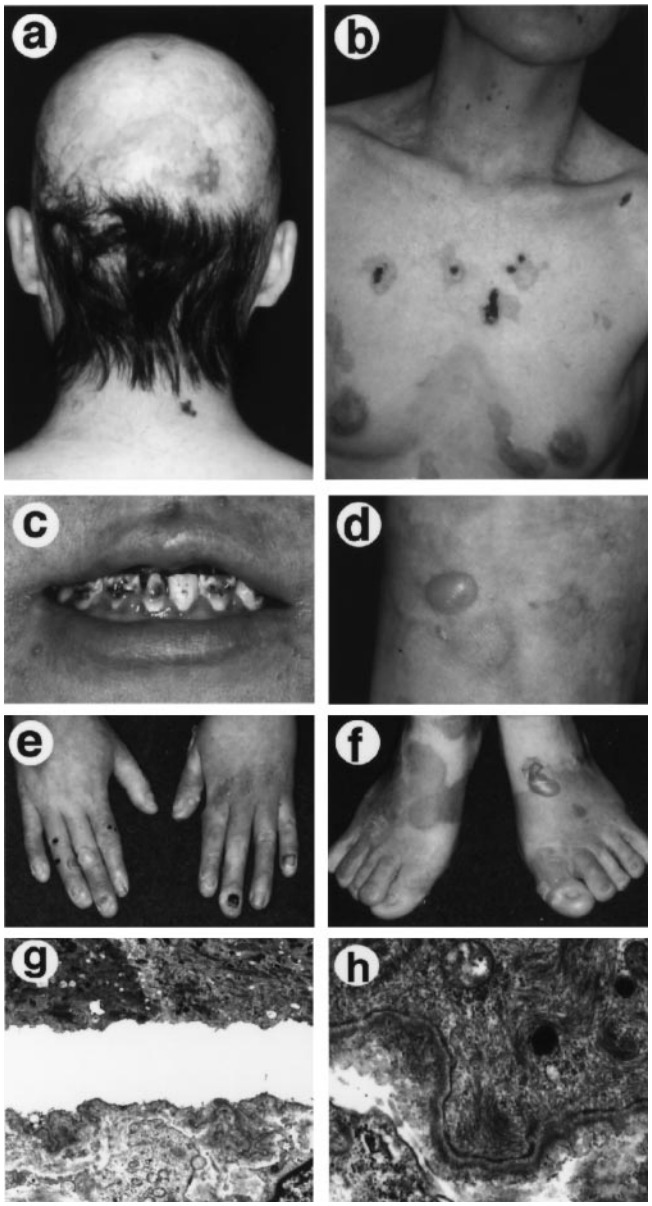

Figure 1. Clinical and ultrastructural features of the proband with GABEB. (*a–f*) Note generalized blistering, scarring alopecia, cigarette paper-like atrophic and depigmented skin, dystrophic nails, and deteriorated dentition. (*g*) Electron microscopy of the edge of the blistered skin demonstrated dermo–epidermal separation within the lamina lucida between the plasma membrane of the basal keratinocytes and the lamina densa. (*h*) The majority of the hemidesmosomes were hypoplastic with thin attachment plaques and the absence of a subbasal dense plate. The shape and number of the anchoring fibrils appeared normal.

Electron microscopy Transmission electron microscopy was performed as previously described (Shimizu *et al*, 1994). In brief, skin specimens from the patient with GABEB, who showed no detectable expression of LAD-1, were fixed with 2% glutaraldehyde and osmium, and embedded in epoxy resin. Semi-thin sections were stained with toluidine blue. Ultrathin sections were cut from the edge of the blister in the same block of tissue, stained with uranyl acetate and lead citrate, and examined with a transmission electron microscope.

Identification and verification of mutations in the COL17A1 gene Mutation analysis of the COL17A1 gene was conducted in the family of the proband with GABEB with absent expression of LAD-1. Genomic DNA was extracted from the peripheral blood of the proband and her parents. The exons and flanking intronic sequences of COL17A1/BPAG2 (GenBank accession no. U76564-U76604) were amplified by polymerase chain reaction (PCR) using genomic DNA as template. The oligonucleotide primers used for the amplification of COL17A1 exons were previously reported (Gatalica *et al*, 1997). Specifically, the following primers were used for amplification of exon 11: the sense primer, 5′-CATTTCTTCTGGTGCTTCTG-3′; the anti-sense primer, 5′-CAGTATGCATGGAAGAAAGG-3′. PCR products were then screened for potential nucleotide substitutions by heteroduplex analysis using conformation-sensitive gel electrophoresis (Ganguly *et al*, 1993). DNA sequencing was carried out with an automatic sequencer (Genetic analyzer 310 A; Perkin Elmer ABI, Foster City, CA).

The mutation identified in the COL17A1 gene was verified from the proband with GABEB and her parents by restriction endonuclease digestion. Specifically, the mutation G258X in exon 11 (see *Results*) created a new restriction enzyme site for the endonuclease *Dde*I.

RESULTS

Expression of LAD-1 and other BMZ epitopes in patients with EB Only one of the 70 patients with GABEB showed entirely negative expression of either LAD-1 or BPAG2. The remaining 69 patients showed positive expression for LAD-1 and BPAG2, whereas the expression of the following cutaneous BMZ molecules was abnormal: in four cases of EB with muscular dystrophy, staining with monoclonal antibody 121 recognizing plectin/HD1 was negative or markedly reduced (Pulkkinen *et al*, 1996). The seven cases of lethal-Herlitz junctional EB were negative for staining with monoclonal antibody GB3 recognizing laminin 5 (Shimizu *et al*, 1994). Staining with GB3 was also reduced in six cases of non-lethal junctional EB, including two cases with the GABEB phenotype. The labeling for LH7.2, which recognizes type VII collagen, was negative in six patients, and markedly reduced in 14 patients with recessive dystrophic EB (Shimizu *et al*, 1996b). Skin from an EB patient with the pyloric atresia was negative for the α6 integrin, whereas expression of the β4 integrin was markedly reduced (Shimizu *et al*, 1996a).

Immunohistochemistry of the skin in the proband with GABEB Immunolabeling for two monoclonal antibodies, MoAb 97–1 and MoAb 97–2 (**Fig 2*A*, *C***), as well as the sera from two patients with linear IgA dermatosis (**Fig 2*E***), proved to be negative in the skin of the proband with GABEB. All three monoclonal antibodies against BPAG2 used in the study also showed negative labeling in the skin of this particular patient (**Fig 2*G***). The skin of this patient expressed all other BMZ components, including laminin 5 (**Fig 2*H***), type VII collagen (**Fig 2*I***), and β4 integrin (**Fig 2*J***), as well as α6 integrin, BPAG1, HD1/plectin, and type IV collagen (data not shown), with findings being comparable with those in normal controls. The use of monoclonal antibody 19-DEJ-1 revealed negative staining for uncein in the skin of all 15 cases of junctional EB (Fine *et al*, 1990).

Blister formation at the lamina lucida with hypoplastic hemidesmosomes Electron microscopy of the edge of the blistered skin taken from the proband with negative LAD-1 and BPAG2 expression demonstrated dermo–epidermal separation within the lamina lucida between the plasma membrane of the basal keratinocytes and the lamina densa (**Fig 1*g***). The majority of the hemidesmosomes were hypoplastic with thin attachment plaques and the absence of a subbasal dense plate (**Fig 1*h***). The shape and number of the anchoring fibrils appeared normal.

Identification and verification of nonsense mutation G258X in COL17A1 To detect any potential mutations in the proband's family, we performed heteroduplex analysis by conformation-sensitive gel electrophoresis, followed by direct automated sequencing of the PCR products spanning the entire coding region of the COL17A1 gene. As shown in **Fig 3(*A*)**, heteroduplex analysis using PCR products that span exon 11 and flanking intronic sequences of the father and the mother demonstrated heteroduplex bands, whereas the proband and an unrelated control showed a homoduplex band only. When the proband's DNA was mixed with control DNA, however, a slower migrating band was noted, suggesting the presence of a homozygous genetic alteration in exon 11 of the proband's COL17A1. Direct sequencing of the PCR products showed that the father and the mother were heterozygous, and the proband was homozygous for a G-to-T transversion in codon 258 of the COL17A1 gene. This mutation substitutes glycine (GGA) by a premature termination codon (TGA), and is designated G258X (**Fig 3*B***). The G258X mutation created a new restriction enzyme site for the endonuclease *Dde*I that was used for verification of this mutation (**Fig 3*C***). In the case of the control DNA (*lane C*), the 218 bp fragment was digested to fragments of 123 bp, 81 bp, and 14 bp. The G-to-T transversion (G258X) created a new enzyme site in the 123 bp fragment, which was further

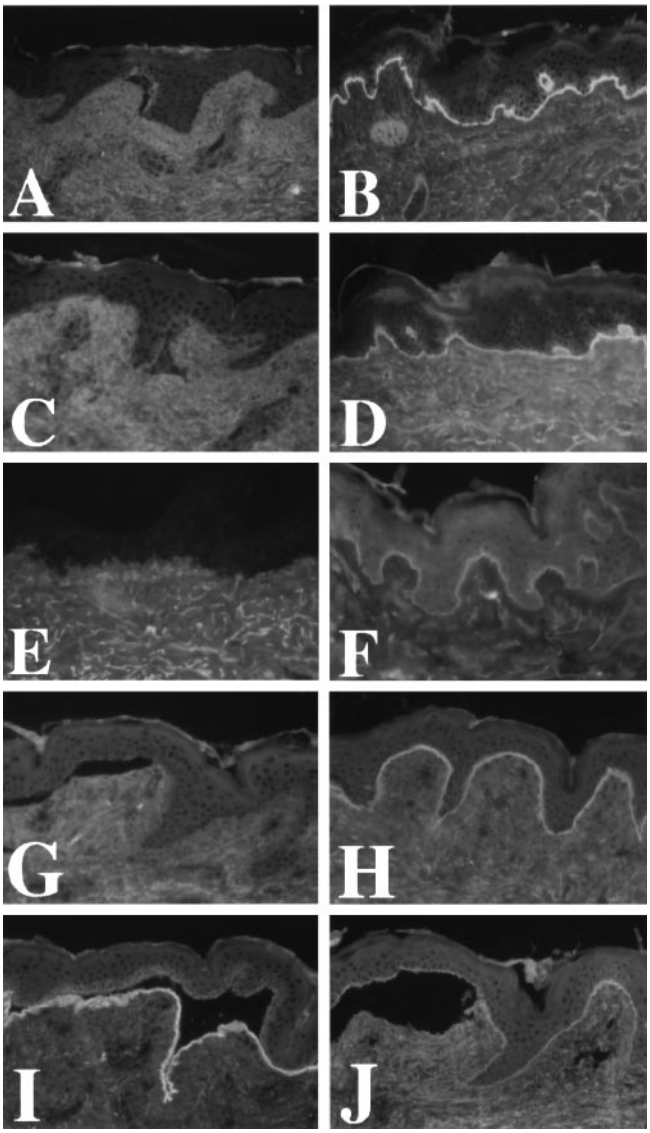

Figure 2. Immunofluorescence of the skin from the proband demonstrated the lack of 97 kDa linear IgA dermatosis antigen (LAD-1). Indirect immunofluorescence of the skin specimen from the proband (*A*, *C*, *E*, *G*, *H*, *I*, *J*) and normal control (*B*, *D*, *F*). Labeling with monoclonal antibodies MoAb 97–1 (*A*) and MoAb 97–2 (*C*), as well as with the serum from a LAD patient (*E*), was negative in the skin of proband with GABEB, whereas normal skin from an unrelated control individual showed positive labeling at the BMZ by these antibodies (*B*, *D*, *F*). Indirect immunofluorescence of the skin of the proband revealed completely negative expression of BPAG2 (*G*; detected by monoclonal antibody D20), whereas the expression of other BMZ molecules, including laminin 5 (*H*; detected by monoclonal antibody GB3), type VII collagen (*I*; detected by monoclonal antibody LH7.2), and β4 integrin (*J*; detected by monoclonal antibody 439–9B), was clearly positive, being comparable with those in normal controls.

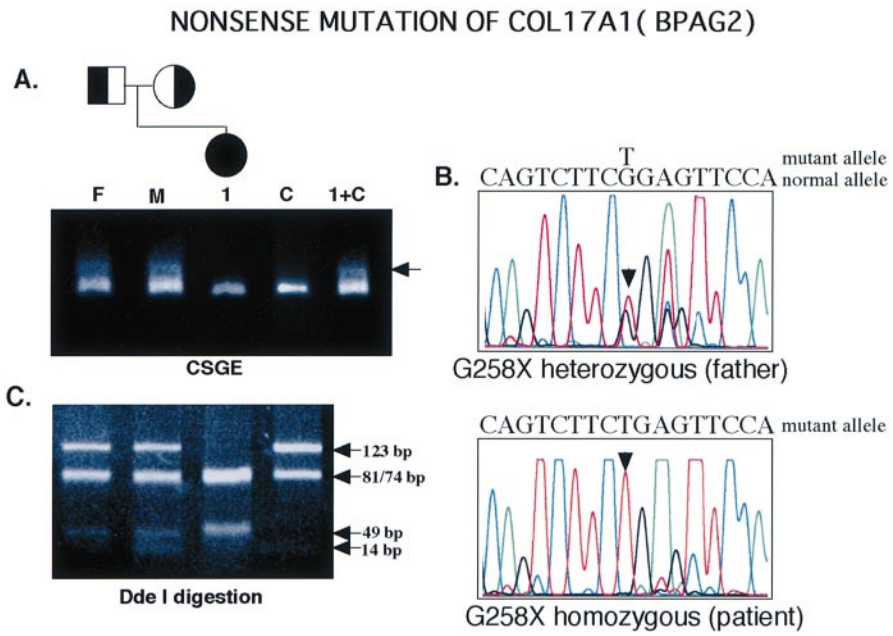

Figure 3. Identification and verification of the nonsense mutation G258X in the COL17A1 gene. (*A*) *Top*, family pedigree; proband with GABEB (1); the father (F) and the mother (M) were clinically unaffected; *bottom*, heteroduplex analysis of the PCR product spanning exon 11 of the COL17A1 gene. When compared with the control (*lane C*), slower migrating bands (*arrow*) were observed with the PCR products from the father (*lane F*) and the mother (*lane M*). The proband's PCR products (*lane 1*) showed a homoduplex band only; however, when control DNA and the proband's DNA were mixed, a heteroduplex band (*lane 1 + C*) similar to that noted in the parents was seen. (*B*) Direct nucleotide sequencing of the exon 11 in the father and the mother (data not shown) revealed a heterozygous G-to-T transversion in codon 258 (G258X mutation) (*arrowhead*). The proband (lower panel) was homozygous for this mutation. (*C*) The presence of this mutation was verified by restriction enzyme digestion. This mutation created a new recognition site for *Dde*I restriction enzyme. In the case of the normal allele (*lane C*), the 218 bp fragment was digested to fragments of 123 bp, 81 bp, and 14 bp. Digestion of the proband's (*lane 1*) DNA revealed that the 123 bp band was further digested to 74 bp and 49 bp fragments, indicating homozygosity for this mutation. The father's (*lane F*) and the mother's (*lane M*) DNA digests showed the presence of 123 bp, 81 bp, 74 bp, 49 bp, and 14 bp bands, indicating that they were heterozygous for this mutation.

digested into fragments of 74 bp and 49 bp. In the proband's DNA, both alleles of 123 bp fragments were digested, and the digestion of the 218 bp PCR product resulted in fragments of 81 bp, 74 bp, 49 bp, and 14 bp. In PCR products from the father (*lane F*) and the mother (*lane M*), only the mutated allele of 123 bp was sensitive to enzyme digestion that yielded five bands: 123 bp, 81 bp, 74 bp, 49 bp, and 14 bp. These results confirmed that the parents were heterozygous, whereas the proband was homozygous, for this G258X mutation.

DISCUSSION

Among the 70 patients with various subtypes of EB evaluated in this study, only one patient, a 63 y old female with non-lethal junctional EB, showed the absence of LAD-1 and COL17A1/BPAG2 expression. The clinical phenotype of this patient, including typical alopecia, atrophic skin, and generalized blistering, corresponds to that of GABEB. While there were three cases with clinical features of GABEB among the patients, the lack of LAD-1 or BPAG2 expression was not detected in the other two cases. Instead, they showed reduced expression of laminin 5, and mutations in the LAMB3 gene have been found in one of these cases (unpublished data). Consistent with these observations is the fact that mutations in laminin 5 genes have been demonstrated in some patients with GABEB (McGrath *et al*, 1995b, 1996c). Absent expression of LAD-1 was previously reported in BPAG2-deficient GABEB patients (Marinkovich *et al*, 1997; Pas *et al*, 1997), and our data further support the notion that the premature termination codon mutations in both alleles of the COL17A1 lead to absent expression of BPAG2, as well as LAD-1, in GABEB patients. Collectively, these findings attest to the heterogeneity of the molecular basis of EB, which now involves specific mutations in 10 different genes of the cutaneous BMZ (Uitto *et al*, 1997).

Using post-embedding immunoelectron microscopy, we have previously demonstrated that COL17A1/BPAG2 is a transmembrane molecule in the hemidesmosome (Ishiko *et al*, 1993), its carboxy terminus extending into the upper lamina densa (Masunaga *et al*, 1997). Thus, BPAG2 may directly connect the intracytoplasmic hemidesmosomal attachment plaque to the lamina densa. We have also shown by immunoelectron microscopy that the target epitope of autoantibodies in patients with linear IgA dermatosis is located within the lamina lucida (Ishiko *et al*, 1996), and the LAD-1 epitopes recognized by monoclonal antibodies MoAb 97–1 and MoAb 97–2 localized to the lamina lucida corresponding to the region between the NC16A domain (plasma membrane) and the carboxy terminal segment (upper lamina densa) of BPAG2 (Ishiko *et al*, 1998). Thus, all these data are consistant with colocalization of the extracellular domain of BPAG2 and LAD-1. Our recent studies revealed that the amino acid sequence of purified LAD-1 is identical to a portion of the extracellular domain of BPAG2 (Zone *et al*, 1998). This finding, together with previous data by others (Marinkovich *et al*, 1997; Pas *et al*, 1997), suggesting a close relationship between BPAG2 and LAD-1, raises the possibility that LAD-1 represents cleaved fragments of BPAG2 or is an alternative spliced product of the same gene, BPAG2/COL17A1. It is not clear why antibodies to LAD-1 do not react with BPAG2 on immunoblot, whereas they react with LAD-1 and the 120 kDa protein (Zone *et al*, 1990; Dmochowski *et al*, 1993; Marinkovich *et al*, 1996; Pas *et al*, 1997). The latter proteins may have unique epitopes that are not present in intact BPAG2 (Pas *et al*, 1997).

The majority of mutations in patients with GABEB reside in the COL17A1 gene (McGrath *et al*, 1995a, 1996a, b; Chavanas *et al*, 1997; Darling *et al*, 1997; Gatalica *et al*, 1997; Jonkman *et al*, 1997; Scheffer *et al*, 1997; Schumann *et al*, 1997; Pulkkinen and Uitto, 1998). The proband of this study, with absent expression of LAD-1 and BPAG2, was shown to be homozygous for a novel nonsense mutation, G258X. This mutation, which is located in exon 11, is predicted to result in synthesis of markedly truncated polypeptides of BPAG2, as well as in the reduced mRNA abundance due to nonsense-mediated mRNA decay (Cui *et al*, 1995), resulting in absent expression of BPAG2 and LAD-1.

Interestingly, previously reported mutations in the COL17A1 gene reside at the extracellular domain of BPAG2 (McGrath *et al*, 1995a, 1996a, b; Chavanas *et al*, 1997; Darling *et al*, 1997; Gatalica *et al*, 1997;

Jonkman *et al*, 1997; Scheffer *et al*, 1997; Schumann *et al*, 1997). The G258X mutation found in our patient is the first mutation located in the intracellular part of BPAG2, thus providing additional clues to the nature of LAD-1. Pas *et al* (1997) showed that the expression of LAD-1 is absent in BPAG2-deficient GABEB patients. Thus they hypothesized that BPAG2 and LAD-1 are closely related. We recently demonstrated that the amino acid sequence of purified LAD-1 is identical to the extracellular portion of BPAG2, and the N-terminal amino end of LAD-1 is located 11 amino acids downstream from the NC16A domain at nucleotide position 1696 in exon 18 (Zone *et al*, 1998). There are several explanations for the close relationship between BPAG2 and LAD-1. One is that LAD-1 is a cleavage product of BPAG2 that exists *in vivo* or that occurs as a result of proteolytic digestion *in vitro*. In the latter case, LAD-1 has no function *in vivo* distinct from BPAG2. Alternatively, LAD-1 could represent alternative splice variants of the same COL17A1 gene and in such a case could serve a structural role in the BMZ. The G258X mutation is located at the nucleotide position 877–879 in exon 11. If LAD-1 is a splice variant of COL17A1, then nucleotide segment 877–879 must be included in the LAD-1 transcript, otherwise we would have had detected normal LAD-1 expression in the skin of our patient. Because the N-terminal amino acid sequence of LAD-1 was located at nucleotide position 1696 in exon 18 (Zone *et al*, 1998), the presence of amino acid 258, encoded by nucleotides 877–879, is unlikely to be seen in LAD-1 molecules, because either 277 amino acid residues in the 97 kDa molecule are absent, or splicing out of exons 12–17 encoding amino acid 279–488 is unlikely to have occurred. Furthermore, if LAD-1 is a splice variant of BPAG2, the mutations in all other patients with absent expression of LAD-1 and BPAG2 have to reside in COL17A1 exons that are expressed in both proteins. Therefore, our data support the hypothesis that LAD-1 is a degradation product, rather than a splice variant of BPAG2. Alternatively, LAD-1 molecule could be a different gene product that colocalizes with BPAG2, and the absence of BPAG2 might lead secondarily to absent expression of LAD-1. This possibility is very unlikely considering the high degree of sequence homology between LAD-1 and the extracellular domain of BPAG2 (Zone *et al*, 1998).

In summary, we have demonstrated that absent expression of LAD-1 in the skin of patients with EB could be observed only in BPAG2-deficient GABEB phenotype, and not in BPAG2-positive GABEB or other EB subtypes. The novel homozygous G258X mutation of COL17A1 is the first mutation residing in the intracellular part of BPAG2. The results support the hypothesis that LAD-1 is a degradation product of BPAG2, rather than a splice variant.

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